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(54) Title: FRAGMENTS OF LEPTIN (OB PROTEIN)

(57) Abstract

A leptin or ob peptide or a functional derivative, analogue or variant thereof, which modulates body weight substantially by means of modulating energy utilisation, a pharmaceutical composition containing such a compound, a process for the preparation of such a compound and the use of such a compound in medicine.

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FRAGMENTS OF LEPTIN (OB PROTEIN)

The invention relates to novel compounds, in particular to novel peptides, to compositions containing such compounds and to the use of such compounds in medicine.

The mechanism of the physiological regulation of energy balance in the body - food intake verses energy output - has been the subject of debate for many years. In a recent publication in Nature Y. Zhang *et al*, (Nature, 372, 425-431, 1994) suggest that one of the molecules which plays a key role in energy balance regulation is the *ob* protein. Zhang *et al* also report the cloning and sequencing of both mouse and human *ob* gene protein or leptin.

The structure of human leptin or (human *ob* protein) and its use in the modulation of body weight in animals is disclosed in United Kingdom Patent application Publication Number GB2292382. This application also discloses certain fragments of leptin which are also stated to be capable of modulating body weight.

Collins et *al in* Nature, Vol 380, page 677, 1996 disclose that the weight reducing properties of leptin may be accounted for by an enhancement of energy utilization as well as decreasing food uptake

We have now discovered certain novel fragments of leptin which surprisingly are indicated to modulate body weight substantially by means of enhancing energy utilization. These fragments are therefore considered to be of particular use in the treatment of nutritional and metabolic disorders, particularly obesity and diabetes.

Accordingly, in a first aspect, the present invention provides a peptide or a functional derivative, analogue or variant thereof, which modulates body weight, substantially be means of modulating energy utilisation.

Preferably the modulation of body weight is a reduction of body weight.

Preferably the modulation of energy utilisation is via an enhancement of energy utilization.

Preferably, the peptide is a fragment of an *ob* protein, especially human *ob* protein, or a functional derivative, analogue or variant thereof.

Hereinafter protein fragments (or peptides) will be referred to with reference to the amino acid sequence of human *ob* protein, using an analogous abbreviation to the following: 'the protein fragment consisting of amino acid residues 1 to 6' is abbreviated to '*ob*1-6'.

Particular peptides include *ob*21 -26 (MVPIQK), *ob27* -32 (VQDDTK), *ob*33 -36 (TLIK) , *ob*37 -41 (TIVTR), *ob*42 -54 (INDISHTQSVSSK), *ob*55 -56 (QK), *ob*57 -74 (VTGLDFIPGLHPILTLSK), *ob*93 -105 (NVIQISNDLENLR), *ob*106 -115 (DLLHVLAFSK), *ob*116 -149

(SCHLPWASGLETLDSLGGVLEASGYSTEVVALSR) and *ob*150-167 (LQGSLQDMLWQLDLSPGC) especially ob57 -74 (VTGLDFIPGLHPILTLSK)

Suitably, the invention includes a peptide formed from any one or more of the aforementioned particular peptides.

Favourably, the invention includes a peptide formed from any of two contiguous members of the aforementioned particular peptides.

As stated, the invention also extends to the functional derivatives, analogues and variants of the peptides mentioned herein:

Functional derivatives includes salts and solvates of the peptides mentioned herein and also the peptides of the invention chemically modified by the attachment of groups or moieties so as to improve the physical properties, such as stability, or the therapeutic properties, for example the pharmacokinetic properties, of the protein.

Functional analogues includes functionally analogous peptides wherein one or more amino acids of the peptides mentioned herein are replaced with alternative amino acids.

Alternative amino acids includes amino acids of alternative stereochemistry to the amino acids in *ob* protein.

Functional analogues also include small molecule agonists or antagonists of the peptides mentioned herein. Such compounds may be prepared and tested according to known procedures, for example those disclosed in GB2292382.

Salts include pharmaceutically acceptable salts, especially pharmaceutically acceptable acid addition salts.

Acid addition salts of the peptides are prepared in a standard manner in a suitable solvent from the parent compound and an excess of an acid, such as hydrochloric, hydrobromic, sulphuric, phosphoric, acetic, maleic, succinic, or methanesulphonic. The acetate salt form is especially useful. Certain of the compounds form inner salts or zwitterions which may be acceptable. Cationic salts are prepared by treating the parent compound with an excess of an alkaline reagent, such as a hydroxide, carbonate or alkoxide containing the appropriate cation. Cations such as Na^+, K^+, Ca^{2+} and NH_4^+ are examples of cations present in pharmaceutically acceptable salts.

Solvates include pharmaceutically acceptable solvates, such as hydrates.

It will be appreciated that the invention includes both peptide and non-peptide compounds.

In addition the invention includes sub-fragments of the particular peptides *ob21*-26, *ob27*-32, *ob33* -36, *ob37*-41 , *ob42* -54 , *ob55*-56, *ob57* -74, *ob93* -105 , *ob106* -115 , *ob116* -149 and *ob150* -167 , especially *ob57 -74* ; or a peptide formed

from any one or more, especially of any two contiguous members, of the said particular peptides; or a functional derivative, analogue or variant thereof.

Suitable peptides or sub-fragments comprise at least 4 amino acids.

The peptides of the invention are suitably prepared by using conventional digestion methods, synthetic techniques or by use of standard expression methodology.

Thus in a further aspect, the present invention provides a process for the preparation of a peptide, or a functional derivative thereof, the process comprising the steps of:

hydrolysing the peptide, especially an *ob* protein and in particular a human *ob* protein, into at least two peptide fragments;

separating the peptide fragments; and optionally thereafter preparing a functional derivative thereof.

The hydrolysis of the protein is suitably effected by enzymic digestion, using for example trypsin.

The separation of the required peptide is conveniently accomplished by use of an appropriate chromatographic means, such as column chromatography.

The specific reaction conditions for the treatment of the *ob* protein, providing they are commensurate with the stability of the required product, are determined by the nature of the particular reagent used, for examples when trypsin is the reagent then the reaction is normally carried out within a temperature range of 25-40°C and a pH range of 7-9, preferably at 37°C and pH 7.4.

As stated, the peptides of the present invention may also be prepared by conventional synthetic procedures, for example by use of liquid or solid-phase peptide synthesis.

Accordingly, the present invention provides a synthetic peptide or a functional derivative, analogue or variant thereof, which modulates body weight, substantially be means of modulating energy utilisation.

Any of the peptides mentioned herein form part of the invention as synthetic peptides.

Peptide bonded units of the proteins associated with the present invention can be prepared by standard peptide synthesis techniques using a peptide synthesiser (Atherton, E. and Sheppard, R.C. (eds.) (1989) Solid Phase Peptide Synthesis: A practical approach, LRL Press, Oxford) followed by procedures appropriate to direct disulphide or amide bond formation.

Methods of well-known peptide synthesis are set forth by Ali et. al., J. Med. Chem., 29:984 (1986) and J. Med. Chem., 30:2291 (1987) and are incorporated by reference herein. Preferably, the peptides are prepared by the solid phase technique of

Merrifield (J. Am. Chem. Soc., 85:2149 (1964)). However, a combination of solid phase and solution synthesis may be used, as in a convergent synthesis in which di-, tri, tetra-, or penta-peptide fragments may be prepared by solid phase synthesis and either coupled or further modified by solution synthesis.

During synthesis, the side chain functional groups (e.g., $-NH_2$, -COOH, -OH, -SH) are protected during the coupling reactions. Normally, the α-amino group is temporarily protected as fluorenylmethoxycarbonyl (Fmoc) but other acid- or base-labile protecting groups can be used, e.g., t-Butoxycarbonyl (Boc). The amino side chain group of lysine is protected as t-butoxycarbonyl, benzyloxycarbonyl or p-chlorobenzyloxycarbonyl (Z or Cl-Z). Acetamidomethyl, trityl, t-butyl, S-t-butyl or para-methylbenzyl (p-MBz) protection is used for cysteines. Hydroxy groups are protected as butyl or benzyl ethers and carboxyl groups are protected as butyl, benzyl (Bz) or cyclohexyl esters.

The peptides can be synthesized either from the C-terminus or the N-terminus, preferably the former. Prior to coupling the alpha-carboxyl group (of a suitable protected amino acid) is activated. One skilled in the art can activate the protected group in a number of ways. For example, one may use N,N' dicyclohexylcarbodiimide (DCC), 2(1H-benzotriazol- 1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate (HBTU), p-nitrophenyl esters (pNp), hydroxybenzotriazole ester (HOBt), N-hydroxy succinimidyl ester (OSu) mixed anhydride or symmetrical anhydride.

Solution synthesis of peptides is accomplished using conventional methods to form amide bonds. Typically, a protected Boc-amino acid which has a free carboxyl group is coupled to a protected amino acid which has a free amino group using a suitable carbodiimide coupling agent, such as N,N' dicyclohexyl carbodiimide (DCC), optionally in the presence of 1-hydroxybenzotriazole (HOBT) and dimethylamino pyridine (DMAP).

In solution phase synthesis, the coupling reactions are preferably carried out at low temperature (e.g., -20°C) in such solvents as dichloromethane (DCM), dimethyl formamide (DMF), N-methyl pyrrolidone (NMP), tetrahydrofuran (THF) acetonitrile (ACN) or dioxane.

If solid phase methods are used, the peptide is built up sequentially starting from the carboxy terminus and working towards the amino terminus of the peptide. Solid phase synthesis begins by covalently attaching the C terminus of a protected amino acid to a suitable resin, such as 4-(2',4'-dimethoxyphenyl-Fmoc-aminomethyl)-phenoxy resin (Rink amide resin, H.Rink, Tetrahedron Letters 28, 3787, (1987)), 4-benzyloxybenzyl alcohol resin (Wang resin, S.S. Wang, JACS, 95, 1328, (1973)) or 4-hydroxymethyl phenoxy acetic acid resin.

In the solid phase synthesis, the first amino acid residue is normally attached to an insoluble polymer. For example, two commonly used polymers are polystyrene (1% cross-linked with divinyl benzene) and 1% cross-linked polyacrylamide. These polymers are functionalised to contain a reactive group, e.g., -OH, $-NH_2$ and $-CH_2Cl$ to link the first amino acid of the targeted peptide (i.e., carboxy terminus). The choice of the linkage between the first amino acid and the polymer is dictated by the carboxy terminus of the peptide. For example, peptides having a carboxyl group at the C-terminus would be linked by an ester linkage and for peptides with a carboxamide ending would have an amide linkage.

Once the first protected amino acid has been coupled to the desired resin, the a amino protecting group is removed by treatment with a secondary amine such as piperidine, and the free carboxyl of the next (protected) amino acid is coupled to this amino group. This process is carried out sequentially, without isolation of the intermediate, until the peptide of interest has been formed. The completed peptide may then be deblocked and/or cleaved from the resin in any order.

Preferred solvents for the coupling reactions include, but are not limited to, dichloromethane (DCM), dimethyl formamide (DMF) and N-methyl pyrrolidone (NMP). After the desired sequence is synthesised, the peptide is deprotected and cleaved from the resin using trifluoroacetic acid or trifluoromethane sulphonic acid.

The preferred method for cleaving a peptide from the support resin is to treat the resin supported peptide with trifluoroacetic acid in the presence of suitable cation and carbonium ion scavengers such as phenol, anisole, thioanisole, ethane dithiol, water or ethylmethyl sulphide.

To obtain the compounds of the present invention, the synthetic peptides may be cyclized/coupled using methods well known in the art.

For example coupling via a disulphide bond of two linear peptides both containing cysteine residues may be achieved in a selective manner by reaction of the free thiol on one chain with a suitably activated cysteine derivative on the other chain. A group which is especially useful as a displaceable protecting group is the S-(carbomethoxy- sulphenyl) derivative. Examplary of this method is the protection of both linear peptides' cysteine residues with the acetamidomethyl (Acm) group. Treatment of one chain with mercury (II) acetate followed by beta mercaptoethanol removes the acetamidomethyl protecting group. Treatment of the second chain with carbomethoxysulphenyl chloride gives the activated species. Stirring of the two peptides in dilute aqueous solution at a pH of about 7 to 8 causes displacement of the carbomethoxysulphenyl group and formation of the interchain disulphide.

If an intramolecular disulphide is to be formed then the corresponding linear peptide can be completely deprotected and produced as a dimercaptan. Any oxidizing

agent known in the art to be capable of converting a dimercaptan to a disulphide may then be used. Examplary of such agents are an alkali metal ferricyanide, (e.g., potassium or sodium ferricyanide), oxygen gas, diiodomethane or iodine. The reaction is conducted in a suitable inert solvent, such as aqueous methanol or water, at temperatures from about 0 to 40°C, under high dilution. The pH is usually maintained at about 7 to 8. Cyclisation may be performed upon the peptide while it is still attached to the support resin or while other functional groups are still protected, but it is preferably performed on the deprotected free peptide.

In cases where two disulphides are to be formed between two linear peptides, two types of cysteine thiol protecting groups can be employed eg Acm and trityl. Each peptide would contain one of each type arranged so that one pair of cysteines to be coupled are protected with trityl groups and the other pair with Acm. Independent removal of the trityl group from each peptide would give two separate monothiol derivatives which can be coupled by activating the thiol on one peptide with 2,2'dipyridyldisulphide and then adding the other monothiol peptide to give the bis(S-acetamido- methyl)disulphide-linked peptide. The second disulphide can be obtained by direct iodine oxidation of this product as described by Kamber (B. Kamber, Helv. Chim. Acta 54, 927, (1971)), and Kamber et. al. (B. Kamber et. al., Helv. Chim. Acta 63, 899, (1980)).

Peptide chains can also be coupled using a linking group such as -$NH(CH_2)_nCO$-. This is most easily achieved by employing the N^a-Fmoc derivative of the corresponding amino acid ($NH_2(CH_2)_nCOOH$) and incorporating it into the growing peptide chain during conventional solid phase synthesis. A similar strategy can be employed to couple peptide chains using the side chain carboxyl of an acidic amino acid such as glutamic acid, and the side chain amino of a basic amino acid such as lysine. In this case compounds such as the N^6-g glutamyllysine derivative below may be incorporated into the growing peptide chain during conventional solid phase synthesis

$CH_3CONH\text{-}CH\text{-}COOH$
|
$(CH_2)_2$
|
CO
|
NH
|
$(CH_2)_4$
|
$FmocNHCHCONH_2$

Coupling to the growing peptide chain is through the a carboxyl of the glutamic acid residue and removal of the Fmoc grouping on the lysine a amino group provides a starting point for addition of further amino acids.

Alternatively the N^a-trityl protecting group may be employed on the glutamic acid residue and after coupling this may be removed with 80% acetic acid and N-acetylated with acetic anhydride. Further couplings may proceed as previously described.

N-terminal N-acetyl groups may be introduced by acetylation of the free amino proteinated by removal of the amino protecting group, with acetic anhydride. C-terminal carboxamide groups are obtained by using an appropriate solid phase synthesis resin such as the Rink amide resin.

As stated the peptides of the invention may also be prepared using recombinant DNA techniques by expression of DNA encoding the polypeptide sequence.

Accordingly, the invention extends to a recombinant peptide or a functional derivative, analogue or variant thereof, which modulates body weight, substantially be means of modulating energy utilisation.

Any of the peptides mentioned herein form part of the invention as recombinant peptides.

In a further aspect, the invention provides a process for preparing a compound according to the invention which process comprises expressing DNA encoding said compound in a recombinant host cell and recovering the product.

The DNA polymer comprising a nucleotide sequence that encodes the compound also forms part of the invention.

The process of the invention may be performed by conventional recombinant techniques such as described in Maniatis et. al., Molecular Cloning - A Laboratory

Manual; Cold Spring Harbor, 1982 and DNA Cloning vols I, II and III (D.M. Glover ed., IRL Press Ltd).

In particular, the process may comprise the steps of:

i) preparing a replicable expression vector capable, in a host cell, of expressing a DNA polymer comprising a nucleotide sequence that encodes said compound;

ii) transforming a host cell with said vector;

iii) culturing said transformed host cell under conditions permitting expression of said DNA polymer to produce said compound; and

iv) recovering said compound.

The invention also provides a process for preparing the DNA polymer by the condensation of appropriate mono-, di- or oligomeric nucleotide units.

The preparation may be carried out chemically, enzymatically, or by a combination of the two methods, in vitro or in vivo as appropriate. Thus, the DNA polymer may be prepared by the enzymatic of appropriate DNA fragments, by conventional methods such as those described by D. M. Roberts et al in Biochemistry 1985, 24, 5090-5098.

The DNA fragments may be obtained by digestion of DNA containing the required sequences of nucleotides with appropriate restriction enzymes, by chemical synthesis, by enzymatic polymerisation on DNA or RNA templates, or by a combination of these methods. Preferably total synthesis of DNA fragments would be employed.

Digestion with restriction enzymes may be performed in an appropriate buffer at a temperature of 20°-70°C, preferably in a volume of 50ml or less with 0.1-10mg DNA.

Enzymatic polymerisation of DNA may be carried out in vitro using a DNA polymerase such as DNA polymerase I (Klenow fragment) in an appropriate buffer containing the nucleoside triphosphates dATP, dCTP, dGTP and dTTP as required at a temperature of 10°-37°C, proteinrally in a volume of 50ml or less.

Enzymatic ligation of DNA fragments may be carried out using a DNA ligase such as T4 DNA ligase in an appropriate buffer at a temperature of 4°C to ambient, in a volume of 50ml or less.

The chemical synthesis of the DNA polymer or fragments may be carried out by conventional phosphotriester, phosphite or phosphoramidite chemistry, using solid phase techniques such as those described in 'Chemical and Enzymatic Synthesis of Protein Fragments - A Laboratory Manual' (ed. H.G. Gassen and A. Lang), Verlag Chemie, Weinheim (1982),or in other scientific publications, for example M.J. Gait, H.W.D. Matthes, M. Singh, B.S. Sproat, and R.C. Titmas, Nucleic Acids Research, 1982, 10, 6243; B.S. Sproat and W. Bannwarth, Tetrahedron Letters, 1983, 24, 5771;

M.D. Matteucci and M.H Caruthers, Tetrahedron Letters, 1980, 21, 719; M.D. Matteucci and M.H. Caruthers, Journal of the American Chemical Society, 1981, 103, 3185; S.P. Adams et al., Journal of the American Chemical Society, 1983, 105, 661; N.D. Sinha, J. Biernat, J. McMannus, and H. Koester, Nucleic Acids Research, 1984, 12, 4539; and H.W.D. Matthes et al., EMBO Journal, 1984, 3, 801. Preferably an automated DNA synthesizer is employed.

The DNA polymer is preferably prepared by ligating two or more DNA molecules which together comprise a DNA sequence encoding the compound.

The DNA molecules may be obtained by the digestion with suitable restriction enzymes of vectors carrying the required coding sequences.

The precise structure of the DNA molecules and the way in which they are obtained depends upon the structure of the desired product. The design of a suitable strategy for the construction of the DNA molecule coding for the compound is a routine matter for the skilled worker in the art.

The expression of the DNA polymer encoding the compound in a recombinant host cell may be carried out by means of a replicable expression vector capable, in the host cell, of expressing the DNA polymer. The expression vector is novel and also forms part of the invention.

The replicable expression vector may be prepared in accordance with the invention, by cleaving a vector compatible with the host cell to provide a linear DNA segment having an intact replicon, and combining said linear segment with one or more DNA molecules which, together with said linear segment, encode the compound, under ligating conditions.

The ligation of the linear segment and more than one DNA molecule may be carried out simultaneously or sequentially as desired.

Thus, the DNA polymer may be preformed or formed during the construction of the vector, as desired.

The choice of vector will be determined in part by the host cell, which may be prokaryotic, such as E. Coli, or eukaryotic, such as mouse C127, mouse myeloma, chinese hamster ovary, fungi e.g. filamentous fungi or unicellular yeast or an insect cell such as Drosophila. The host cell may also be in a transgenic animal. Suitable vectors include plasmids, bacteriophages, cosmids and recombinant viruses derived from, for example, baculoviruses or vaccinia.

The preparation of the replicable expression vector may be carried out conventionally with appropriate enzymes for restriction, polymerisation and ligation of the DNA, by procedures described in, for example, Maniatis et al., cited above. Polymerisation and ligation may be performed as described above for the preparation of the DNA polymer. Digestion with restriction enzymes may be performed in an

appropriate buffer at a temperature of 20°-70°C, proteinrally in a volume of 50ml or less with 0.1-10mg DNA.

The recombinant host cell is prepared, in accordance with the invention, by transforming a host cell with a replicable expression vector of the invention under transforming conditions. Suitable transforming conditions are conventional and are described in, for example, Maniatis et al., cited above, or "DNA Cloning" Vol. II, D.M. Glover ed., IRL Press Ltd, 1985.

The choice of transforming conditions is determined by the host cell. Thus, a bacterial host such as E. coli may be treated with a solution of $CaCl_2$ (Cohen et al, Proc. Nat. Acad. Sci., 1973, 69, 2110) or with a solution comprising a mixture of RbCl, $MnCl_2$, potassium acetate and glycerol, and then with 3-[N-morpholino]-propane-sulphonic acid, RbCl and glycerol. Mammalian cells in culture may be transformed by calcium co-precipitation of the vector DNA onto the cells.

The invention also extends to a vector comprising a compound of the invention.

The invention also extends to a host cell transformed with a replicable expression vector of the invention.

Culturing the transformed host cell under conditions permitting expression of the DNA polymer is carried out conventionally, as described in, for example, Maniatis et al and "DNA Cloning" cited above. Thus, preferably the cell is supplied with nutrient and cultured at a temperature below 45°C.

The expression product is recovered by conventional methods according to the host cell. Thus, where the host cell is bacterial, such as E. coli it may be lysed physically, chemically or enzymatically and the protein product isolated from the resulting lysate. If the product is to be secreted from the bacterial cell it may be recovered from the periplasmic space or the nutrient medium. Where the host cell is mammalian, the product may proteinrally be isolated from the nutrient medium.

The DNA polymer may be assembled into vectors designed for isolation of stable transformed mammalian cell lines expressing the product; e.g. bovine papillomavirus vectors or amplified vectors in chinese hamster ovary cells (DNA cloning Vol.II D.M. Glover ed. IRL Press 1985; Kaufman, R.J. et al., Molecular and Cellular Biology 5, 1750-1759, 1985; Pavlakis G.N. and Hamer, D.H., Proceedings of the National Academy of Sciences (USA) 80, 397-401, 1983; Goeddel, D.V. et al., European Patent Application No. 0093619, 1983).

The peptides prepared by use of the above mentioned methods can, as required, be purified by a number of techniques. Preferred embodiments include gel filtration, chromatogaphy, reverse phase HPLC and crystallisation, especially chromatogaphy is used. The purified products can then be analysed for purity using

HPLC, amino acid analysis, amino acid sequencing and fast atom bombardment and/or electrospray mass spectrometry.

The functional derivatives, analogues and variants of the proteins mentioned herein may be prepared by using conventional methods analogous to those mentioned herein.

As stated, the compounds of the invention are indicated to have useful pharmaceutical properties. Accordingly, there is also provided a compound of the invention for use as an active therapeutic substance.

In particular the compounds of the invention are considered to be capable of modulating body weight substantially by means of enhancing energy utilization and are therefore of potential use in the treatment of nutritional and metabolic disorders, particularly obesity and diabetes.

The invention also provides a method for the treatment of nutritional and metabolic disorders, which method comprises the administration of an effective, pharmaceutically acceptable and non-toxic amount of a compound of the invention.

The invention therefore further provides a pharmaceutical composition comprising a compound of the invention and a pharmaceutically acceptable carrier.

In use the active compound will normally be employed in the form of a pharmaceutical composition in association with a human or veterinary pharmaceutical carrier, diluent and/or excipient, although the exact form of the composition will depend on the mode of administration. The active compound may, for example, be employed in the form of tablets, capsules, lozenges or syrups for oral administration; in the form of snuff, aerosol or nebulisable solution for inhalation; in the form of sterile solutions for parenteral administration, or in the form of creams, lotions, liniments, gels, ointments or sprays for topical administration. Parenteral routes of administration include intravenous, intramuscular, subcutaneous, transcutaneous and intraperitoneal administration.

Also included are formulations of the above derivatives suitable for use in subcutaneously implanted pumps or controlled release devices, in transdermal patches and as micronised powders suitable for intranasal administration.

The dosage ranges for administration of the compounds of the present invention are those to produce the desired effect on the condition to be treated, the dosage will proteinrally vary with age, extent or severity of the medical condition and contraindications, if any. The dosage can vary from 0.001mg/kg/day to 50mg/kg/day, but preferably 0.01 to 1.0mg/kg/day.

Solid oral dosage forms may contain conventional excipients such as diluents, for example lactose, microcrystalline cellulose, dicalcium phosphate, mannitol, magnesium carbonate, glycine, dextrose, sucrose, starch, mannitol, sorbitol

and calcium carbonate; binders, for example liquid glucose, syrup, acacia, gelatin, starch mucilage, methylcellulose, polyvinylpyrrolidone, alginates, and pregelatinised starch; disintegrants for example starch, alginic acid, microcrystalline cellulose, pectin, cross-linked polyvinylpyrrolidone, sodium starch glycollate and sodium carboxymethyl-cellulose; glidants for example talc and silica; lubricants for example stearic acid and magnesium stearate; preservatives for example sorbic acid and methyl or propyl parahydroxybenzoate, or pharmaceutically acceptable wetting agents for example sodium lauryl sulphate.

Capsules consist of a shell, normally of gelatin together with other ingredients for example, glycerol, sorbitol, surface-active agents, opaque fillers, preservatives, sweeteners, flavours and colours. The contents of capsules may include diluents, lubricants and disintegrants. Tablets consist of compressed powders or granules, may be coated or uncoated and may be designed so as to dissolve, disperse or effervesce before administration to the patient, or to dissolve or disperse in the gastrointestinal tract either immediately after swallowing, or, for example in the case of tablets with acid-insoluble coatings, at later times. Tablets usually contain excipients such as diluents, binders, disintegrants, glidants, lubricants and may contain colours and flavours. Effervescent tablets proteinrally contain acids together with carbonates or bicarbonates. Coatings for tablets may consist of natural or synthetic resins, gums, insoluble fillers, sugars, plasticisers, polyhydric alcohols and waxes and may also contain colours and flavours. Lozenges and pastilles are intended to dissolve in the mouth. Lozenges may be moulded or compressed, and usually have a flavoured base. Pastilles are moulded from a base of gelatin and glycerol or acacia and sucrose. They may contain a preservative as well as colours and flavours.

Film-coating resins include cellulose derivatives, zein, vinyl polymers and acrylic resins, and coating compositions usually include plasticisers, such as castor oil or glycerol triacetate. Enteric-coating resins include cellulose acetate phthalate and copolymers of methacrylic acid.

Solid compositions suitable for oral administration may be obtained by conventional methods of blending, filling, granulation, tabletting or the like. Repeated blending operations may be used to distribute the active agent throughout those compositions employing large quantities of fillers.

Liquid compositions suitable for oral administration may be in the form of, for example, elixirs, mixtures, concentrated solutions, suspensions, emulsions or linctuses. They may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid compositions may contain conventional excipients such as suspending agents, for example sucrose, sorbitol, gelatin, methyl cellulose, carboxymethylcellulose, hydroxypropyl methyl cellulose, sodium alginate,

Xanthan gum, acacia, carageenan, silica, aluminium stearate gel; emulsifying agents, for example lecithin, acacia, sorbitan mono-oleate; aqueous or non-aqueous vehicles which include edible oils, oily esters, for example esters of glycerol, ethanol, glycerol; buffering agents for example citrates and phosphates of alkali metals; preservatives, for example sodium benzoate, sorbic acid, methyl or propyl parahydroxybenzoate; and if desired, conventional flavouring and colouring agents.

The composition may be implanted subcutaneously, for example in the form of a compressed tablet or slow release capsule.

Alternatively, compositions suitable for injection may be in the form of solutions, suspensions or emulsions, or dry powders which are dissolved or suspended in a suitable vehicle prior to use.

Fluid unit dosage forms are prepared utilising the compound and a pyrogen-free sterile vehicle. The compound, depending on the vehicle and concentration used, can be either dissolved or suspended in the vehicle. Solutions may be used for all forms of parenteral administration, and are particularly used for intravenous infection. In preparing solutions the compound can be dissolved in the vehicle, the solution being made isotonic if necessary by addition of sodium chloride and sterilised by filtration through a sterile filter using aseptic techniques before filling into suitable sterile vials or ampoules and sealing. Alternatively, if solution stability is adequate, the solution in its sealed containers may be sterilised by autoclaving. Advantageously additives such as buffering, solubilising, stabilising, preservative or bactericidal, suspending or emulsifying agents and/or local anaesthetic agents may be dissolved in the vehicle.

Dry powders which are dissolved or suspended in a suitable vehicle prior to use may be prepared by filling pre-sterilised drug substance and other ingredients into a sterile container using aseptic technique in a sterile area. Alternatively the drug and other ingredients may be dissolved in an aqueous vehicle, the solution is sterilised by filtration and distributed into suitable containers using aseptic technique in a sterile area. The product is then freeze dried and the containers are sealed aseptically.

Parenteral suspensions, suitable for intramuscular, subcutaneous or intradermal injection, are prepared in substantially the same manner, except that the sterile compound is suspended in the sterile vehicle, instead of being dissolved and sterilisation cannot be accomplished by filtration. The compound may be isolated in a sterile state or alternatively it may be sterilised after isolation, e.g. by gamma irradiation. Advantageously, a suspending agent for example polyvinylpyrrolidone is included in the composition to facilitate uniform distribution of the compound.

In a further aspect there is provided a method of treating nutritional and metabolic disorders, which comprises administering to the sufferer an effective, non-toxic amount of a compound of the invention.

The invention also provides the use of a compound of the invention for the manufacture of a medicament for treating nutritional and metabolic disorders, such as obesity and diabetes.

No unexpected toxicological effects are expected when compounds of the invention are administered in accordance with the present invention.

The following examples illustrate compounds of the invention.

Pharmacological Methods: The activity of the compounds of the invention are assessed according to the methodology set our below:

EFFECT OF LEPTIN FRAGMENTS ON FOOD INTAKE IN SD RATS

Surgery

Rats are pre-treated with Synulox (0.1ml/100g) approx 1 hour before anaesthesia, and then anaesthetised with Domitor (0.04ml/100g i.m.) and sublimase (0.9ml/100g i.p.) Each rat has a cannula implanted stereotaxically into the lateral brain ventricle under sterile conditions. Anaesthesia is then reversed using Antisedan and Nubain (50% v/v : 50% v/v 0.02ml/100g) I.P. After surgery each rat receives 0.05 ml Zenecarp.

Experimental procedure:

Experiment 1 : Following surgery the body weight of each animal was monitored daily throughout the procedure.
In order to verify that the cannula was in the lateral ventricle, Angiotensin II (100ng/5μl) was injected icv and water intake was monitored for 5 min after injection.
24 hour food intake was measured on day 5 and 6 after surgery. On day 6 the animals were divided according to their body weight into 3 groups (a,b and c, 8 rats per group) and then fasted overnight.. On the day of experiment (day 7) rats were injected icv as follows::
group a-vehicle (PBS, phosphate buffer solution, 5μl /rat);
group b- human leptin (11.5 μg/5μl); and
group c- leptin tryptic digest (30μg/5μl).
A known quantity of food in excess of the daily requirement was supplied to the rats immediately after the icv injection procedure was completed.. Food intake and body weight were then measured 24h later.. The results obtained are shown in Table 1.

Experiment 2: A separate group of animals was prepared exactly as described above but on the day of experiment after overnight fast , groups of rats were injected as follows:
group a-vehicle (PBS 5μl /rat);
group b-human leptin (8.75 μg/5μl);
group c-murine leptin (10 μg/5μl);
group d-ob 57 -74 , sequence VTGLDFIPGLHPILTLSK (3.33μg/5μl);
Again a pre-weight quantity of food, in excess of the daily requirement, was re-given following the injection; change in body weight and the quantity of food consumed were recorded 24h later. The results obtained are shown in Table 2.

Results:

Table 1. Effect of human leptin and its fragments given intracerebroventricularly on body weight and food intake in SD rats. * $P<0.05$.

	24h food intake (g)	Bwt Change (g/24h)
Vehicle (5µl/rat, n=8)	36.68 ±2.5	31.44 ± 1.2
h-Leptin (11.5 µg/rat, n=8)	30.62±1.5*	22.88 ± 2.05*
h-Leptin tryptic digest (30 µg/rat, n=8)	34.6 ± 1.99	23.88 ± 1.2*

Table 2. Effect of human leptin (h-leptin), murine leptin (m-leptin) and leptin fragment 57-74 (VTGLDFIPGLHPILTLSK) given intracerebroventricularly on body weight and food intake in SD rats. * $P<0.05$.

	24h food intake (g)	Bwt Change (g/24h)
Vehicle (5µl/rat, n=8)	32.16 ±0.8	30.55 ± 0.67
h-Leptin (8.75 µg/rat, n=8)	33.7±1.6	24.5 ± 3.7
m-Leptin (10 µg/rat, n=8)	31.3 ± 1.7	23.7 ± 2.18*
Leptin fragment 57-74 (3.33 µg/rat, n=8)	33.6 ± 0.9	26.8 ± 1.2*

Claims:

1. A peptide or a functional derivative, analogue or variant thereof, which modulates body weight, substantially be means of modulating energy utilisation.

2. A peptide or a functional derivative, analogue or variant thereof, which reduces body weight, substantially be means of modulating energy utilisation.

3. A peptide according to claim 1 or claim 2, wherein the peptide is a fragment of an *ob* protein, or a functional derivative, analogue or variant thereof.

4. A peptide according to any one of claims 1 to 3, selected from a fragment of human *ob* protein in the list: *ob*21 -26 , *ob27* -32 , *ob*33 -36 , *ob*37 -41 , *ob*42 -54 , *ob*55 -56, *ob*57 -74, *ob*93 -105 , *ob*106 -115, *ob*116 -149 and *ob*150-167.

5. A peptide according to any one of claims 1 to 4, wherein the peptide has the amino acid sequence VTGLDFIPGLHPILTLSK.

6. A peptide according to claim 1, formed from one or more of the peptides of claim 4.

7. A peptide according to claim 1, formed from two contiguous members of the peptides of claim 4.

8. A synthetic or recombinant peptide, being a peptide according to any one of claims 1 to 7.

9. A nucleotide sequence that encodes a peptide of any one of claims 1 to 7.

10. A vector comprising a nucleotide sequence that encodes a peptide of any one of claims 1 to 7.

11. A host cell transformed with a replicable expression vector of claim 9.

12. A process for the preparation of a peptide according to claim 1, or a functional derivative thereof, the process comprising the steps of:
hydrolysing the peptide into at least two peptide fragments;

separating the peptide fragments; and optionally thereafter preparing a functional derivative thereof.

13. A process for preparing a peptide according to any one of claims 1 to 7, which process comprises expressing DNA encoding said peptide in a recombinant host cell and recovering the product.

14. A process accoring to claim 13, comprising the steps of:

i) preparing a replicable expression vector capable, in a host cell, of expressing a DNA polymer comprising a nucleotide sequence that encodes the required peptide;

ii) transforming a host cell with said vector;

iii) culturing said transformed host cell under conditions permitting expression of said DNA polymer to produce said peptide; and

iv) recovering said peptide.

15. A pharmaceutical composition comprising a compound according to claim1, and a pharmaceutically acceptable carrier.

16. A compound according to claim 1, for use as an active therapeutic substance.

17. A compound according to claim 15, for use in the treatment of nutritional and metabolic disorders.

18. A method for the treatment of nutritional and metabolic disorders, which method comprises the administration of an effective, pharmaceutically acceptable and non-toxic amount of a compound according to claim 1.

19 The use of a compound according to claim1, for the manufacture of a medicament for treating nutritional and metabolic disorders.